

# Differential Supply of Autochthonous Organic Carbon and Nitrogen to the Microbial Loop in the Delaware Estuary

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**ABSTRACT:** Using stable isotope tracer techniques in 4-h bottle incubations, the importance of organic matter transfer from phytoplankton to heterotrophic bacteria (bacteria) has been re-evaluated in the Delaware Estuary, considering carbon (C) and nitrogen (N) cycles separately. The hypothesis is that the transfer of C and N from phytoplankton to bacteria varies both temporally and spatially along estuarine gradients in response to variation in factors such as terrestrial organic C supply, inorganic N speciation and concentrations, and extracellular release of dissolved organic matter by phytoplankton. The percentage of autochthonous dissolved organic C being assimilated by bacteria varied between 3% and 10% of primary production and was not related to the rate of primary production. The transfer of N was considerably more variable when compared to C transfer, averaging ca. 20% of phytoplankton N assimilation; individual experiments yielded rates as high as 50%. Unlike C, autochthonous dissolved organic N transfer appears to vary with the magnitude of primary production, and its assimilation by bacteria accounted for 0–56% of the total measured bacterial N uptake. The results highlight the importance of separate consideration of C and N elemental cycles in evaluating sources of organic matter to the estuarine microbial loop.

## Introduction

The fate of organic matter produced during photosynthesis, either by transfer to higher trophic levels through the grazer food chain, assimilation by heterotrophic bacteria via the dissolved organic matter (DOM) pool, or respired during transfer in either pathway, has important consequences for energy and material flow in marine systems. In the open ocean, where bacterial production is wholly dependant on autochthonous DOM, it appears that roughly 50% of organic carbon (C) produced through primary production is used to fuel bacterial production (Ducklow 2000). Because of analytical difficulties in identifying and characterizing the components of the DOM pool that are important for bacterial production, and the fact that the flux of DOM into bacteria is rapid (limiting any accumulation of the most labile compounds), questions regarding the link of phytoplankton and heterotrophic bacteria via the DOM pool have relied on relating indirect measurements of standing stocks and production rates (Wright 1984). In estuaries, these estimates are less reliable because bacterial production is fueled by a combination of autochthonous and allochthonous DOM. Differentiating between the two sources is difficult.

Tight linkages between phytoplankton and bacterial production have been documented in the Delaware Estuary, United States, on certain spatial and temporal scales (Coffin and Sharp 1987;

Cifuentes 1991; Hoch and Kirchman 1993) even with large anthropogenic inputs of DOM. These observations suggest bacteria may play a significant role in directing the flow of organic C and nitrogen (N) produced by phytoplankton in some estuaries. The covariation in phytoplankton and bacteria would suggest that bacterial production is, in part, limited by the supply of autochthonous dissolved organic carbon (DOC). The rate of DOC assimilated relative to primary production (percent transfer) may provide insight into what might be controlling the supply of DOC to bacteria (Hoch and Kirchman 1993).

The transfer of autochthonous DOM to heterotrophic bacteria is dependent on the rate of DOM production. Direct release of DOM by phytoplankton may account for 10–25% of the total flux to the DOM pool (Williams 1990; Nagata 2000) but there is still uncertainty as to the factors that control direct release (Fogg 1983; Williams 1990; Mykkestad 1995). Several experiments have shown that, under conditions of nutrient limitation and sufficient light, phytoplankton will release high C : N ratio DOM in an overflow mode (Fogg 1983; Wood and Van Valen 1990; Mykkestad 1995). A second proposed model of direct release hypothesizes that phytoplankton leak a small, fixed percentage of DOM, including low molecular weight material such as dissolved free amino acids and other nitrogenous compounds (Mykkestad et al. 1989; Bronk and Glibert 1993). Both models are needed to explain observations of direct release of DOC and dissolved organic nitrogen (DON).

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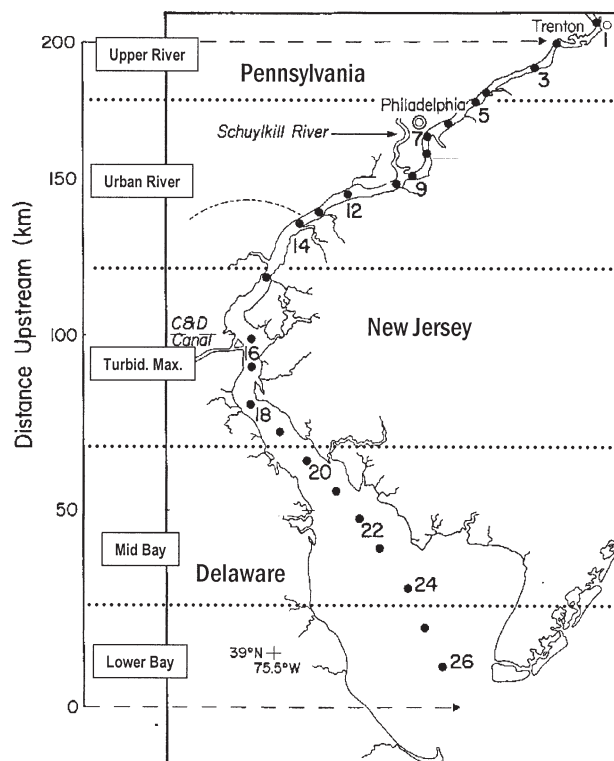


Fig. 1. Map of Delaware Estuary with geographically fixed sampling stations. Distance scale at left (km) is from the mouth of the estuary at Cape Henlopen, Delaware, and Cape May, New Jersey, to 210 km upstream at Trenton, New Jersey.

While C and N may be supplied to bacteria at different rates, studies concerned with quantifying sources to the microbial loop often rely on measurements of primary and bacterial production with assumed C : N ratios, in order to link phytoplankton and bacteria in C and N cycles. As a result, C and N cycles may not be clearly resolved. Stable isotope tracer techniques, using both  $^{13}\text{C}$  and  $^{15}\text{N}$  have been used successfully to understand the dynamics of phytoplankton C and N uptake in single bottle experiments (Dauchez et al. 1995; Legendre and Gosselin 1996; Bury et al. 1995). By employing size fractionation, dual isotope tracer techniques can be used to more directly quantify

the transfer of C and N from phytoplankton to bacteria.

The goal of this research was to re-evaluate phytoplankton and bacterial linkages along a nutrient gradient in a eutrophic estuarine system. Using dual labeling with  $^{13}\text{C}$  and  $^{15}\text{N}$ , I attempted to directly trace phytoplankton uptake, DOM release, and subsequent assimilation by bacteria. The hypothesis is that the transfer of organic matter from phytoplankton to heterotrophic bacteria will vary along estuarine gradients.

## Methods and Materials

### STUDY SITE

Experiments were conducted in the Delaware Estuary during five cruises in 2002. Using our 25-yr database, five regions of the estuary have been defined based primarily on concentrations of inorganic N, DOM, and rates of primary production (Sharp unpublished data) (Fig. 1). Representative values for the five regions can be illustrated with data from the June 2002 cruise (Table 1). Stations 1–6 represent the freshwater, upper river region. These stations are characterized by relatively high allochthonous inputs of inorganic N and DOM and relatively low primary production. The urban river region (stations 7–14) has the highest input of inorganic N and DOM, primarily from sewage and industrial sources in Philadelphia, Pennsylvania, Camden, New Jersey, and Wilmington, Delaware. Despite high concentrations of inorganic N and only moderate turbidity, primary production is generally low, except for some periods during the summer (Pennock and Sharp 1986). Stations 15–20 comprise the turbidity maximum region, where production is severely light limited due to high concentrations of suspended sediments (Sharp et al. 1982). South of the turbidity maximum, the Delaware River broadens into the mid bay region (stations 21–24), characterized by some of the highest areal primary production and chlorophyll *a* (chl *a*) concentrations in the estuary (Pennock and Sharp 1986). High primary production in this region is the result of an increased light field and sufficient nutrient supply advected from upstream.

TABLE 1. Selected biogeochemical parameters at each of the five regions of the Delaware Estuary during June 2002. Regions are defined primarily by differences in dissolved organic matter, dissolved inorganic nitrogen, and light attenuation.

	Distance to the Capes (km)	DIN			DON ( $\mu\text{M}$ )	Light attenuation $-k$ ( $\text{m}^{-1}$ )	Areal production ( $\mu\text{M C m}^2 \text{d}^{-1}$ )	Chl <i>a</i> ( $\mu\text{g chl l}^{-1}$ )
		$\text{NO}_3 + \text{NO}_2$ ( $\mu\text{M}$ )	$\text{NH}_4$ ( $\mu\text{M}$ )	DOC ( $\mu\text{M}$ )				
Upper river	170–210	38.8	6.1	259	23	1.76	15.2	1.4
Urban river	130–160	60.1	10.9	291	28	1.57	34.2	3.6
Turbidity maximum	70–90	52.7	3.9	223	38	2.27	14.8	2.5
Mid bay	30–50	20.9	2.9	176	35	1.11	259.6	18.2
Lower bay	0–20	0.1	0.3	130	15	0.69	109.5	11.1

The lower bay region, found between stations 25 and 26, is generally N limited, with moderate to high primary production and low chl *a* concentrations (Pennock 1985; Pennock and Sharp 1994).

#### SAMPLE COLLECTION AND ROUTINE BIOGEOCHEMICAL ANALYSES

Sampling was conducted along the main axis of the Delaware Estuary on board the R/V *Cape Henlopen*. Samples were collected 1 m below the surface in 10-l Niskin bottles on a rosette sampler fitted with a CTD system. Temperature and salinity profiles were made at each station where samples were collected. Light attenuation profiles of photosynthetically active radiation (PAR) were also determined using a submersible quantum meter (Biospherical Instruments). Samples were immediately filtered, collected in acid-washed 125-ml (HDPE) bottles and quick frozen in dry ice for later determination of inorganic nutrient concentrations ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ ,  $\text{SiO}_2$ ). Nutrients were analyzed colorimetrically (Strickland and Parsons 1972) with modifications described by Sharp et al. (1982). Chl *a* was measured fluorometrically (Strickland and Parsons 1972). Filters prepared for chl *a* were extracted in 40 : 60 DMSO : acetone to allow faster extraction and analysis. DOC and total dissolved nitrogen (TDN) samples were filtered through GF/F filters and collected in baked (450°C, 24 h) 20-ml glass ampoules and stored frozen until analysis. DOC analysis was performed on either Shimadzu TOC 5000 or TOC-V high temperature combustion instruments (Sharp et al. 2004). TDN analysis was completed either by the persulfate oxidation-colorimetric method (Solorzano and Sharp 1980) or Shimadzu TOC-V (Sharp et al. 2004). Sharp et al. (2004) found that persulfate and high temperature combustion methods were generally comparable with persulfate oxidation yielding somewhat higher estimates of TDN. Dissolved inorganic carbon (DIC) was measured using the Monterrey Bay Research Institute-clone DIC analyzer with acid-sparging and non-dispersive infrared (NDIR) analysis (Walz and Friederich 1996).

#### BACTERIAL PRODUCTION

During selected experiments, bacterial production was estimated using  $^3\text{H}$ -leucine (Kirchman et al. 1985) under saturating concentrations (20 nM). Triplicate 1.5-ml samples, as well as a kill control (160  $\mu\text{l}$  50% trichloroacetic acid [TCA]), were prepared for each station. Incubations were carried out using the microcentrifuge method (Smith and Azam 1992; Kirchman 2001) for 30 min in the dark at ambient seawater temperature. Estimates of bacterial

production were made in all regions of the estuary during spring, summer, and fall. Leucine incorporation was converted to C units assuming 1.5 kg C to 1 mol leucine ratio (Ducklow 2000). Bacterial N demand was calculated using a bacterial C : N ratio of 4.5 (Goldman and Dennett 1991).

#### STABLE ISOTOPE TRACER EXPERIMENTS

In order to evaluate C and N cycling between phytoplankton and bacteria, two size fractionation treatments were used in conjunction with stable isotope enrichment experiments. Previous work in the Delaware Estuary has shown that phytoplankton and bacteria can be relatively well discriminated using a 1.0- $\mu\text{m}$  size separation (Coffin and Sharp 1987; Lebo 1990; Preen and Kirchman 2004). Unmanipulated, whole community samples were collected in order to quantify bacterial C and N uptake from phytoplankton DOM release. A second treatment was created by removing the phytoplankton size fraction ( $> 1.0 \mu\text{m}$ ) before inoculation with stable isotopes. This bacterial control treatment was prepared in order to assess the direct uptake of inorganic C and N by the bacterial size fraction. A comparison of DOC and TDN samples from both whole community and bacterial control treatments immediately following the filtration step verified that there was no enrichment of the DOM pool from cell lysis due to filtration. Samples for isotope uptake were prepared in 1-l acid-washed HDPE bottles.

Once both whole community and bacterial control bottles were prepared, stable isotope tracers were added. To one set of whole community and bacterial control bottles,  $^{13}\text{C}$ -bicarbonate and  $^{15}\text{N}$ -ammonium were added. To the other set of bottles,  $^{13}\text{C}$  and  $^{15}\text{N}$ -nitrate were added. The additions were made to approximate a 10% concentration enrichment based on expected DIC and nutrient concentrations. Sample bottles were incubated in deck incubators with flowing surface seawater to maintain ambient temperature. Samples were incubated for 4 h at 85% of incident light with all incubations carried out between 0900 and 1600 each day. Results from incubations completed under varying light intensities (ca. 4–100% of surface PAR) suggested that there was no enhanced organic matter transfer from phytoplankton to bacteria due to the light intensity, i.e., high DOM loss by phytoplankton due to light shock (data not shown).

Upon terminating the incubations, phytoplankton and bacterial size fractions were collected for particulate organic matter (POM) and isotopic analysis. This required post incubation fractionation for the whole community treatment. A volume of sample water (50–200 ml) was filtered through a 47-mm, 1.0- $\mu\text{m}$  polycarbonate filter under a vacuum ( $< 250 \text{ mm Hg}$ ). The  $< 1.0\text{-}\mu\text{m}$  filtrate was collect-

ed in an acid-washed vacuum flask and filtered onto a baked (400°C, 4 h) 25-mm GF/F filter, taking care to adequately rinse the sides of the vacuum flask. The >1.0- $\mu\text{m}$  particulate, which was retained on the polycarbonate filter, was then backwashed onto a separate 25-mm GF/F filter using filtered rinse water of similar salinity. A sample from the bacterial control treatment was filtered onto a third GF/F filter. Each GF/F filter was immediately placed in a 12  $\times$  5 mm washed (methylene chloride and acetone rinsed) tin cup and stored in a desiccator until isotopic analysis. Duplicate filters were prepared for both size classes from whole community and bacterial control treatments. It has been estimated that >50% of heterotrophic bacteria may be retained on GF/F filters in the Delaware Estuary (Cottrell personal communication) though this was not tested specifically during this study.

In order to determine whether there was any significant isotope blank as a result of abiotic absorption (inorganic particle association or adsorption or  $^{13}\text{C}$  or  $^{15}\text{N}$  dissolved inorganic constituent being retained within the filter due to inadequate rinse), a time zero bottle was collected and immediately filtered after isotopic addition. No measurable enrichment was detected. During several late fall (October–November) and early spring cruises (early March) stations were assessed for rates of primary production. During these periods there was essentially no measurable primary production determined by  $^{13}\text{C}$  or  $^{14}\text{C}$  incubations. These cruises offered an ideal situation to evaluate interactions between  $^{13}\text{C}$  and  $^{15}\text{N}$  isotopes and inorganic particles. The agreement between C uptake estimates based on  $^{13}\text{C}$  and  $^{14}\text{C}$  under these conditions and no apparent enrichment in the particulate fraction over 4–6 h suggest that abiotic capture of isotope was not significant.

Particulate organic carbon (POC) and nitrogen (PON) concentrations, as well as isotope enrichment, were determined using a Europa Tracermass isotope ratio mass spectrometer (IRMS) system. Samples were stored for at least 1 wk prior to combustion to ensure that they were completely dry. After analysis, both specific uptake rate,  $V_n$  ( $\text{h}^{-1}$ ), and transport rate,  $\rho_n$  ( $\mu\text{mol l}^{-1} \text{h}^{-1}$ ), were determined based on the equations of Dugdale and Wilkerson (1986).

$$V_n = \frac{d(\text{at}\% \text{P}^{15}\text{N})}{dt} \times \frac{1}{\text{at}\% \text{DI}^{15}\text{N}} \times \frac{1}{h} \quad (1)$$

where  $d(\text{at}\% \text{P}^{15}\text{N})/dt$  is the accumulation of heavy isotope in the particulate fraction over the course of the incubation and  $\text{at}\% \text{DI}^{15}\text{N}$  is the enriched pool of inorganic N at the beginning of the incubation.

The three calculated C and N uptake rates (>1.0  $\mu\text{m}$  from whole community, <1.0  $\mu\text{m}$  from whole community, and <1.0 in bacterial control) were used to differentiate between phytoplankton uptake, autochthonous DOM uptake by bacteria, and direct uptake of inorganic N (also see results for bicarbonate uptake in this fraction) by bacteria. Independent analysis of parallel  $^{14}\text{C}$  and  $^{13}\text{C}$  tracer experiments show that calculated  $^{13}\text{C}$  uptake in the >1.0- $\mu\text{m}$  fraction was comparable to  $^{14}\text{C}$  primary production estimates in the Delaware Estuary (Parker 2004). By subtracting the uptake rate found in the bacterial control from uptake rates in the whole community <1.0- $\mu\text{m}$  fraction, the autochthonous DOM uptake could be estimated. All reported uptake rates in the whole community <1.0- $\mu\text{m}$  fraction have been corrected for direct uptake of DIN by bacteria.

#### MODEL FOR INTERPRETATION OF $^{13}\text{C} : ^{15}\text{N}$ UPTAKE RATES

The model used for interpreting the tracer results for  $^{13}\text{C}$  and  $^{15}\text{N}$  uptake experiments is based on the assumption that there is efficient separation of phytoplankton and bacteria based on size. During the course of the incubation it is assumed that phytoplankton will access both  $\text{HCO}_3^-$  and inorganic N (the atom% [at%] of the inorganic substrates were calculated based on the measured concentration of inorganic substrate [DIC,  $\text{NH}_4^+$ , or  $\text{NO}_3^-$ ], with an assumed natural abundance value, and the tracer addition of 99 at%, Isotec, Co.). The inorganic C and N are rapidly assimilated by the phytoplankton with isotopic enrichment of POM occurring within 18 min (Fig. 2). After a lag period of approximately 30 min, isotopic enrichment of the bacterial size fraction begins. This lag period reflects the time required for labeled DOM production to occur. DOM may be produced from various sources including sloppy feeding and grazing (Jumars et al. 1989), zooplankton excretion, and viral lysis (Fuhrman and Noble 1995); it is assumed that on time scales of 2–6 h the dominant mode of DOM production is through direct release from phytoplankton.

Two potential models were considered for the isotopic composition of the DOM used by bacteria. The first model was based on the assumption that the isotopic enrichment of phytoplankton DOM produced over the course of the incubation would be equal to the isotopic enrichment of the phytoplankton POM at any given time. Using this model, calculated uptake by bacteria are significantly (ca. >500%) higher than C uptake rates calculated for phytoplankton. This result is due to relatively low values in the denominator of Eq. 1. These unreasonably high bacterial uptake estimates

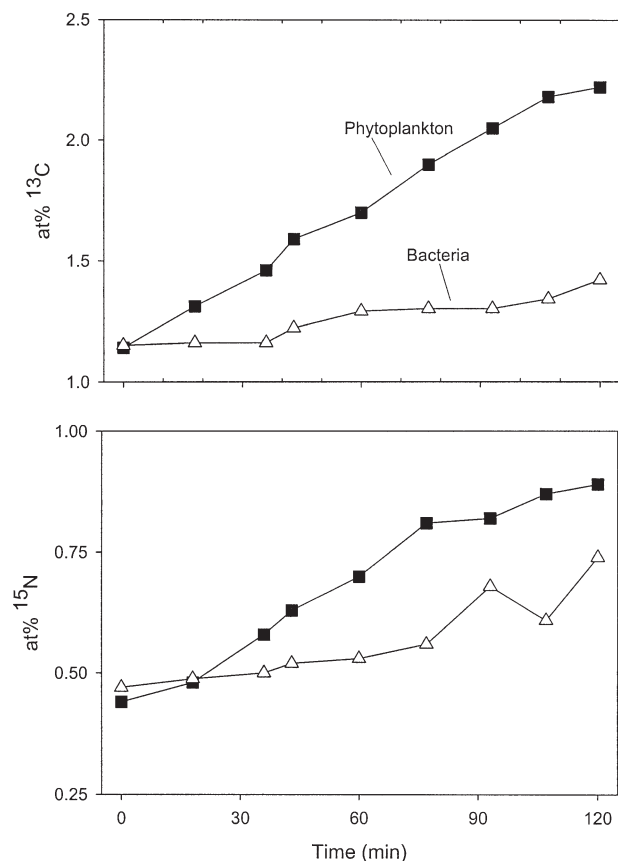


Fig. 2. Time series of isotopic carbon and nitrogen particulate organic matter (POM) enrichment for phytoplankton and bacteria after addition of  $^{13}\text{HCO}_3$  and  $^{15}\text{NH}_4$ . Phytoplankton POM becomes enriched within 22 min followed by bacterial POM enrichment after 36 min.

indicate that the isotopic enrichment in phytoplankton DOM produced during the incubation is not equal to isotopic enrichment in the phytoplankton POM. The compounds being released are not in equilibrium with the phytoplankton POM.

The second model used for the isotopic enrichment of DOM assumes that phytoplankton DOM produced over the course of the experiment was in equilibrium with the inorganic substrates being traced for phytoplankton (Table 2). This assumption suggests that the DOM produced during the incubation (and subsequently assimilated by bacteria) represented immediate release products, which presumably are in equilibrium with the inorganic pool. Calculated bacterial C uptake rates based on assumptions result in rates that are comparable to net bacterial production rates based on tritiated leucine assays and are on the order of 3–15% of phytoplankton uptake. These rates are consistent with our current understanding of phytoplankton and bacterial rates.

## Results

Chl *a* concentrations varied significantly with season and location in the Delaware Estuary (Table 3). The highest concentrations were generally found in the mid bay region during spring ( $29.3 \mu\text{g chl l}^{-1}$ ) and early summer ( $7.5 \mu\text{g chl l}^{-1}$ ). Atypical for the Delaware, the highest chl *a* concentration found in March ( $10.4 \mu\text{g chl l}^{-1}$ ) was in the turbidity maximum region. The highest primary production estimates (using both  $^{13}\text{C}$  and  $^{14}\text{C}$  tracer techniques) was also observed at this location. Relatively low chl *a* concentrations were found in all seasons in the urban region of the Delaware Estuary despite the availability of inorganic nutrients.

Chl *a* concentrations in the  $>1.0\text{-}\mu\text{m}$  size fraction were compared to a separate estimate of total chl *a* to assess the separation of phytoplankton and bacteria and to determine the effectiveness of backwashing and recovering the  $>1.0\text{-}\mu\text{m}$  size class after fractionation. Percent recovery of chl *a*, determined from 34 individual samples was  $99.5\% \pm 12$ , indicating that the majority of the chl *a* was found in the  $>1.0\text{-}\mu\text{m}$  fraction and that the  $>1.0\text{-}$

TABLE 2. Results of bacterial uptake rate measurements based on  $^{13}\text{C}$  tracer enrichment and assuming the isotopic enrichment of DOM is equivalent to the isotopic composition of phytoplankton POM (model A) or the tracer isotopic enrichment of the inorganic pool (model B). Calculated bacterial carbon uptake based on each model is presented as a percentage of phytoplankton carbon uptake.

Time	Particulate Organic Matter Enrichment		Models of Bacterial Substrate Enrichment		Phytoplankton Uptake	Bacterial Uptake		Bacterial Production as a Percentage of Phytoplankton	
	Bact at% $^{13}\text{C}$	Phyto at% $^{13}\text{C}$	Model A $^{13}\text{C-HCO}_3$	Model B Phyto $^{13}\text{C}$		Model A nmol $\text{C l}^{-1} \text{h}^{-1}$	Model B nmol $\text{C l}^{-1} \text{h}^{-1}$	Model A	Model B
0	1.15	1.14	1.14	20.00					
18	1.16	1.31	1.31	20.00	708	3922	21	554	3
36	1.16	1.46	1.46	20.00	667	1042	11	156	2
43	1.22	1.59	1.59	20.00	785	4341	62	553	8
60	1.29	1.70	1.70	20.00	700	5000	89	714	13
77	1.30	1.90	1.90	20.00	740	3076	74	416	10
93	1.30	2.05	2.05	20.00	734	2127	61	290	8
107	1.34	2.18	2.18	20.00	729	2049	68	281	9
120	1.42	2.22	2.22	20.00	675	2500	86	370	13

TABLE 3. Chlorophyll *a* and particulate carbon (C) and nitrogen (N) concentrations in phytoplankton (>1.0  $\mu\text{m}$ ) and bacterial (<1.0  $\mu\text{m}$ ) size fractions in five regions of the Delaware Estuary, and significant values (*t*-test) for differences in average C : N ratios of phytoplankton and bacterial size fractions within each estuary region.

Region	Month	Chlorophyll <i>a</i> ( $\mu\text{g l}^{-1}$ )		Particulate Carbon ( $\mu\text{M}$ )		Particulate Nitrogen ( $\mu\text{M}$ )		Average C : N Ratio for Region ( $\pm$ SD)		p value
		> 1 $\mu\text{m}$	< 1 $\mu\text{m}$	> 1 $\mu\text{m}$	< 1 $\mu\text{m}$	> 1 $\mu\text{m}$	< 1 $\mu\text{m}$	> 1 $\mu\text{m}$	< 1 $\mu\text{m}$	
Upper river	June	0.9	bd	42.7	13.1	4.2	1.4			
	October	0.3	bd	40.9	21.2	2.6	1.2	13.0 (3.8)	13.3 (5.7)	0.376
Urban river	June	3.5	bd	66.9	14.7	6.6	1.4			
	August	3.5	bd	110.4	12.1	11.4	1.1			
Turbidity maximum	October	0.5	bd	48.7	23.7	3.8	2.2	10.9 (1.6)	10.9 (0.6)	0.457
	March	10.4	bd	59.5	18.6	7.8	1.2			
Mid bay	June	1.9	bd	68.4	18.5	6.4	1.7			
	August	2.4	0.1	39.6	25.3	5.9	2.5			
	October	0.8	bd	65.5	14.1	5.5	1.2	9.2 (2.5)	12.0 (2.2)	0.113
	March	2.2	bd	27.5	15.5	3.1	1.8			
	June	29.3	1.9	87.8	24.3	13.2	3.3			
Lower bay	August	7.5	0.3	57.2	24.1	7.0	2.3			
	October	1.5	bd	24.8	20.3	2.2	1.8	8.7 (1.9)	9.4 (1.7)	0.158
	March	0.8	bd	37.4	15.3	3.9	1.5			
	June	6.3	0.4	54.1	24.4	7.2	3.5			
	August	2.9	0.2	29.1	15.6	3.9	2.4			
	October	3.3	bd	20.4	16.5	1.9	1.8	8.8 (1.7)	8.3 (1.8)	0.136

$\mu\text{m}$  particles could be effectively recovered from the polycarbonate filter surface. Chl *a* concentrations in the <1.0- $\mu\text{m}$  fraction were also used to evaluate the effectiveness of size fractionation (Table 3). In most cases, the chl *a* concentrations in the <1.0- $\mu\text{m}$  size fraction were below limits of detection (0.1  $\mu\text{g chl l}^{-1}$ ). The highest chl *a* concentration in the <1.0- $\mu\text{m}$  size fraction (1.9  $\mu\text{g chl l}^{-1}$ ) was found in the mid bay region in June. In all cases, chl *a* in the <1.0- $\mu\text{m}$  fraction was at most between 4% and 6% of the total chl *a* at any station. This low percentage of chl *a* may be attributed to a slight shift to smaller cells in summer.

$^{13}\text{C}$  enrichment from  $\text{H}^{13}\text{CO}_3$  uptake in the bacterial control treatments provide another means of evaluating the effectiveness of size fractionation in separating autotrophic and heterotrophic cells. Generally, there was little or no measured  $^{13}\text{C}$  enrichment in the bacterial control treatments. In cases where there was measured enrichment, the calculated bicarbonate uptake never exceeded 5% of C uptake in the >1.0- $\mu\text{m}$  fraction. Assessment of  $^{13}\text{C}$  enrichment in the bacterial control treatments, in conjunction with chl *a* concentrations, suggests that the contribution of autotrophic cells to <1.0- $\mu\text{m}$  activity was not significant and rates measured in this fraction may be interpreted as representative of bacterial activity.

The fraction of POC and PON in the two size classes varied in each region of the Delaware Estuary (Table 3). POC in the <1.0- $\mu\text{m}$  size fraction accounted for 20–30% of the total POC in the upper river, urban river, and turbidity maximum regions. In the mid and lower bay regions, POC in

the <1.0- $\mu\text{m}$  size fraction represented 40–60% of total POC. These results indicate that roughly half of the biomass available for higher trophic levels was found in the <1.0- $\mu\text{m}$  size fraction in the lower regions of the Delaware Estuary, and contributed at most, one third of the biomass in the upper regions.

The particulate C : N ratio was similar for the two size fractions (Table 3). Compared to the Redfield ratio, particulate C : N was high in the upper and urban river regions (11–13) and lower in the mid and lower bay regions (8–9). In the turbidity maximum region, the <1.0- $\mu\text{m}$  size fraction was relatively enriched in C (C : N ratio of 12.0) compared to the >1.0- $\mu\text{m}$  size fraction (C : N ratio of 9.2) although these differences were not significant (*t*-test,  $p > 0.05$ ). The patterns observed in the C : N ratio of the POM were probably due to differences in the percentage of detrital material contributing to the POM. In the upper regions of the Delaware Estuary, POM had more of a detrital influence compared with the lower regions where the C : N ratios reflect a larger phytoplankton and bacterial signature (approached the Redfield ratio).

C assimilation rates were calculated for the >1.0- $\mu\text{m}$  and <1.0- $\mu\text{m}$  size classes during spring, summer, and fall in the salinity gradient of the estuary (Fig. 3). During March, C assimilation by the >1.0- $\mu\text{m}$  size fraction was highest in the turbidity maximum region. The highest C uptake rates in the >1.0- $\mu\text{m}$  fraction were found in June and August. During that time, peaks were found in the mid bay region and decreased dramatically at the lower bay region. In addition to the mid bay productivity peak in August, a second productivity

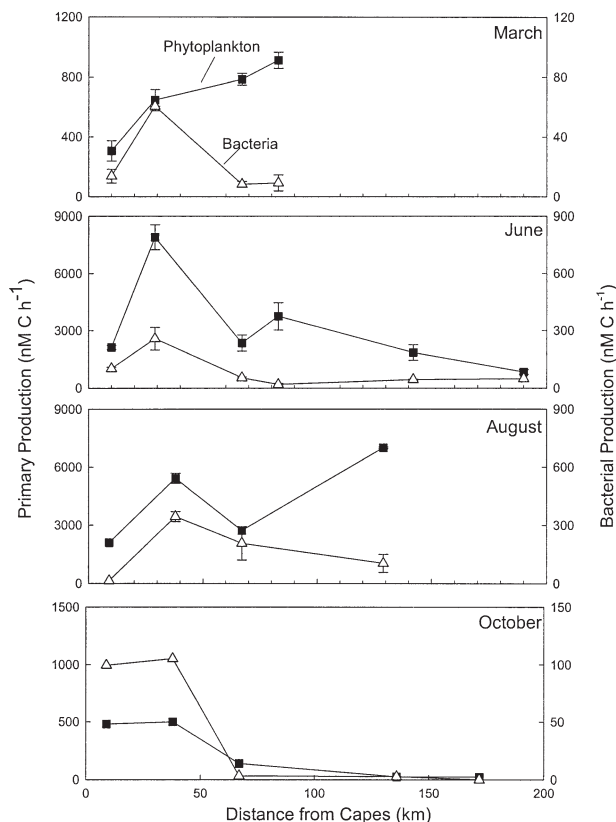


Fig. 3. Carbon uptake along the length of the Delaware Estuary. Carbon uptake in the bacterial size fraction represents only  $^{13}\text{C}$  taken up and released by phytoplankton and assimilated by bacteria over the course of the incubation (autochthonous dissolved organic carbon). Each plot is from a single cruise in 2002: March, June, August, and October.

maximum, with the highest measured C uptake rate for the entire transect, was found at the urban river region. During October, rates were comparable to those found in March, with uniformly low C uptake in the upper three regions of the estuary and increasing at the mid and lower bay regions to a maximum value of ca.  $500 \text{ nmol l}^{-1} \text{ h}^{-1}$ .

C uptake in the  $<1.0\text{-}\mu\text{m}$  size class was low in the upper regions of the estuary and increased in the mid and lower bay regions during each cruise (Fig. 3). C uptake did not follow trends of uptake observed in the  $>1.0\text{-}\mu\text{m}$  fraction in the upper three regions of the estuary. This was particularly apparent in August, when, despite high C uptake in the  $>1.0\text{-}\mu\text{m}$  fraction in the urban river region, there was little C uptake (1% of  $>1.0 \mu\text{m}$  uptake) in the  $<1.0\text{-}\mu\text{m}$  fraction at that time. In spring and summer, C uptake in the  $<1.0\text{-}\mu\text{m}$  fraction represented at most 10% of the uptake in the  $>1.0\text{-}\mu\text{m}$  size fraction, and those rates were only seen in the mid and lower bay regions. In October, C uptake in the  $<1.0\text{-}\mu\text{m}$  fraction was low in the upper regions

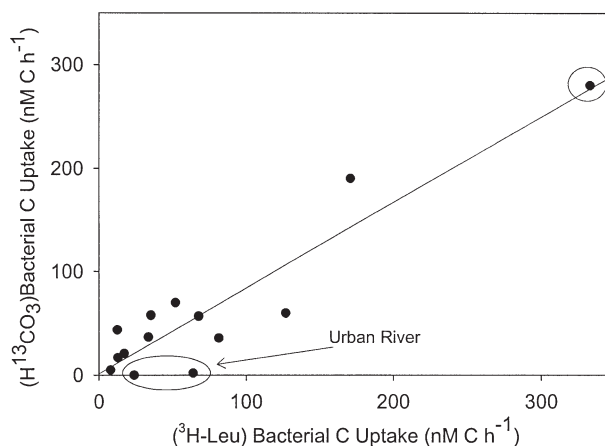


Fig. 4. Bacterial carbon uptake estimated by  $^3\text{H}$ -leucine incorporation (reported in carbon units) versus  $^{13}\text{C}$  uptake in the  $>1.0\text{-}\mu\text{m}$  size fraction. The linear regression (excluding the highest uptake rate circled) resulted in a slope of 0.83 ( $r = 0.93$ ,  $n = 13$ ).

and increased dramatically in the mid and low bay regions. C uptake in the  $<1.0\text{-}\mu\text{m}$  fraction was as high as 20% of C uptake in the  $>1.0\text{-}\mu\text{m}$  size class in those regions.

Total bacterial production (reported in C units) estimated from tritiated leucine incorporation versus  $^{13}\text{C}$  uptake estimates for the  $<1.0\text{-}\mu\text{m}$  fraction were compared in 14 individual bottle experiments (Fig. 4). Bacterial production and  $^{13}\text{C}$  uptake were similar (slope = 0.83,  $R = 0.93$ ,  $n = 13$ ) even after removing the highest bacterial carbon estimates (in circle). In two experiments, both from the urban river region, there was measured bacterial production assayed by  $^3\text{H}$ -leucine ( $24\text{--}64 \text{ nmol C h}^{-1}$ ) and no measured C uptake by  $^{13}\text{C}$  in the  $<1.0\text{-}\mu\text{m}$  fraction.

A comparison of bacterial N uptake in  $<1.0\text{-}\mu\text{m}$  whole community and bacterial control treatments was made for each region of the estuary (Fig. 5). Similar to patterns of C uptake, the highest N assimilation in the  $<1.0\text{-}\mu\text{m}$  size fraction was generally found in the mid and lower bay regions. In the mid bay, N uptake in the  $<1.0\text{-}\mu\text{m}$  fraction was always significantly higher than N uptake in the bacterial controls. In the lower bay, uptake in the  $<1.0\text{-}\mu\text{m}$  fraction and bacterial controls were similar. In the upper river, urban river and in the turbidity maximum regions there was little or no uptake in the  $<1.0\text{-}\mu\text{m}$  fraction, but N uptake in the bacterial control was substantial. There were two instances in March and August when there was measured N uptake in the  $<1.0\text{-}\mu\text{m}$  fraction in the turbidity maximum and urban river regions. During August, significant N uptake occurred in the  $<1.0\text{-}\mu\text{m}$  fraction in the urban river region; this occurred during the peak in primary production. At this time,

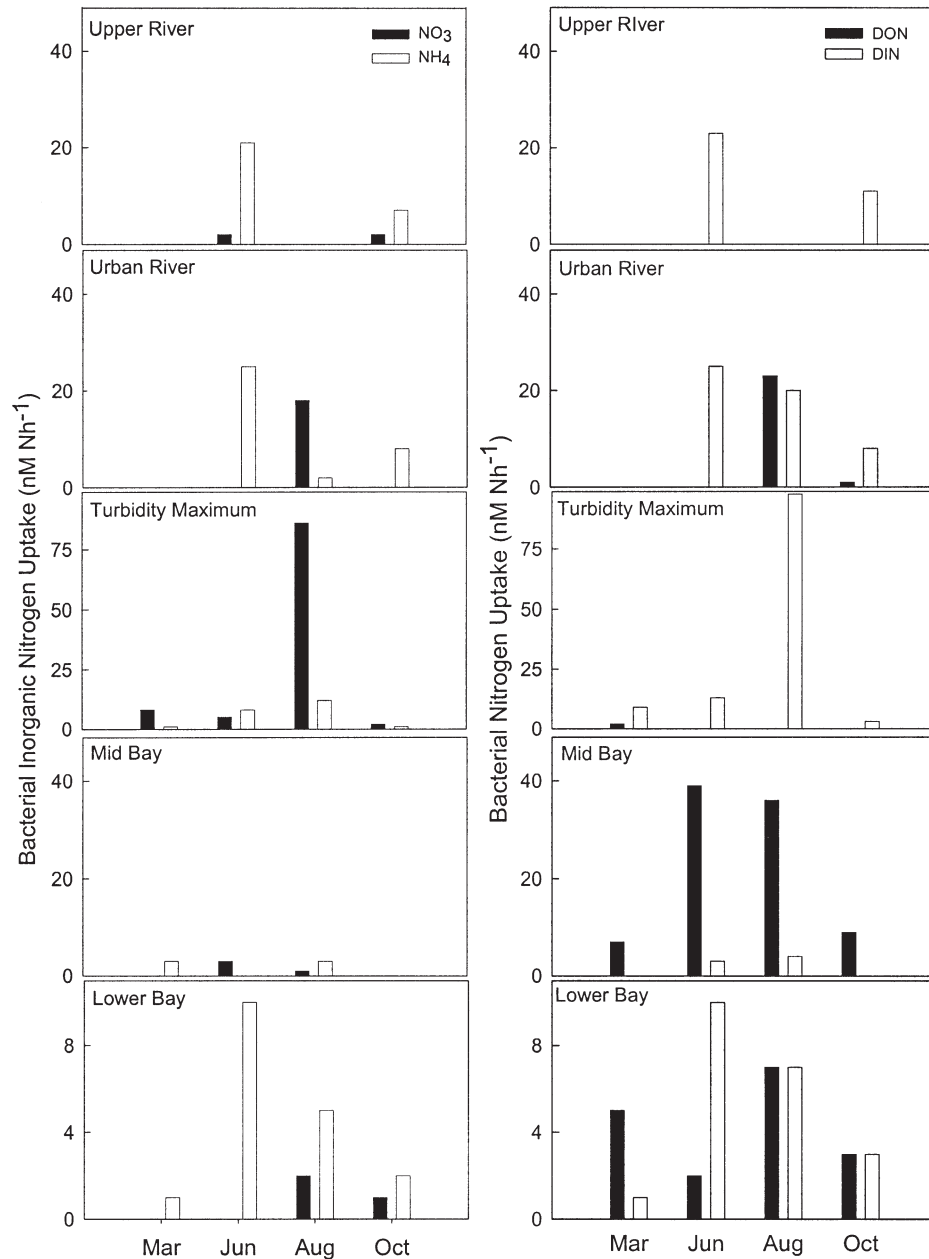


Fig. 5. Dissolved inorganic (DIN =  $\text{NH}_4^+ + \text{NO}_3^-$ ) and organic nitrogen (DON) uptake by bacteria. DON uptake in the bacterial size fraction represents only the fraction of  $^{15}\text{N}$  taken up and released by phytoplankton during the course of the incubation. Inorganic nitrogen ( $\text{NH}_4^+$  or  $\text{NO}_3^-$ ) utilization by bacteria in each of the five regions of the Delaware Estuary. Phytoplankton were removed from the system before the incubations were started. Note that the scales for different regions vary.

N uptake measured in the  $<1.0\text{-}\mu\text{m}$  fraction and N uptake in the bacterial control were roughly equivalent.

Uptake of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in the bacterial control treatment was observed in all regions of the Delaware Estuary (Fig. 5). In the upper and urban river as well as the mid and lower bay regions,  $\text{NH}_4^+$  uptake accounted for 60–100% of the total in-

organic N uptake in the bacterial control. During the August peak in primary production in the urban river region,  $\text{NO}_3^-$  uptake in the bacterial control represented 90% of total DIN uptake, as the ambient  $\text{NH}_4^+$  concentration at that station approached zero. In contrast to the other regions,  $\text{NO}_3^-$  uptake in the bacterial control treatment was high in the turbidity maximum region; values up to

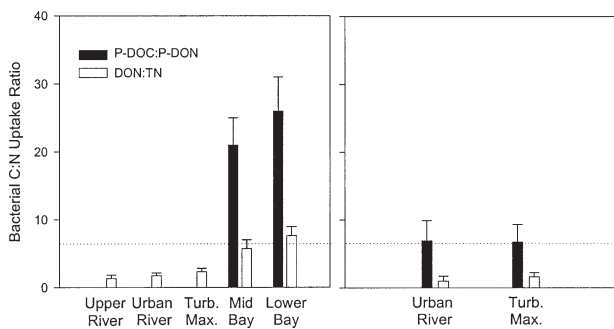


Fig. 6. General bacterial uptake ratios of phytoplankton DOC: DON (P-DOC: P-DON) compared with bacterial uptake of phytoplankton DOC to total nitrogen (DON + DIN). The Redfield ratio of 6.6 is included as a reference of assumed phytoplankton carbon and nitrogen uptake requirements. Specific cases with P-DOC transfer in the urban river (August) and turbidity maximum region (March).

87% of total inorganic N uptake were measured during spring and summer. In the mid bay region, there did not appear to be a preference for either  $\text{NH}_4^+$  or  $\text{NO}_3^-$  in the bacterial control treatment, and the total N assimilation ( $\text{NH}_4^+ + \text{NO}_3^-$ ) in the bacterial control treatments was lower than N assimilation in the  $<1.0\text{-}\mu\text{m}$  fraction (at most 10% of N uptake in the  $<1.0\text{-}\mu\text{m}$  fraction). In the lower bay region,  $\text{NH}_4^+$  uptake in the bacterial control was significantly higher than  $\text{NO}_3^-$  uptake.

The average C : N uptake ratio for the  $<1.0\text{-}\mu\text{m}$  size class was calculated for the five regions of the estuary (Fig. 6). Because there was no measured N uptake in the  $<1.0\text{-}\mu\text{m}$  fraction in the upper three regions, no ratio is reported. In the mid bay and lower bay regions, the C : N uptake ratio was relatively high (21 : 1 to 25 : 1) compared to the Redfield ratio (6.6 : 1). By including the N uptake measured in the bacterial control (i.e.,  $<1.0\text{-}\mu\text{m}$  C uptake to  $<1.0\text{-}\mu\text{m}$  N uptake + bacterial control N uptake), the C : N uptake ratios in the mid bay and lower bay regions were 5.7 and 7.6, respectively. In the upper regions of the estuary, the C : N ratio of  $<1.0\text{-}\mu\text{m}$  C to bacterial control N uptake was 1.3 to 2.3. Separate analysis of C : N uptake ratios was made for the two cases where there was appreciable N uptake in the  $<1.0\text{-}\mu\text{m}$  fraction in the upper regions. In both cases, the C : N uptake ratio in the  $<1.0\text{-}\mu\text{m}$  fraction was comparable to the Redfield ratio (7.6 and 6.9 in the urban river and turbidity maximum regions, respectively); with the addition of N uptake in the bacterial control, the C : N uptake ratio decreased to 1.4 and 2.3 for the urban river and turbidity maximum regions, respectively.

The uptake of C and N by the  $<1.0\text{-}\mu\text{m}$  fraction (expressed as a percentage of uptake in the  $>1.0\text{-}\mu\text{m}$ ) was similar in the upper three regions of the estuary and became dissimilar in the mid and

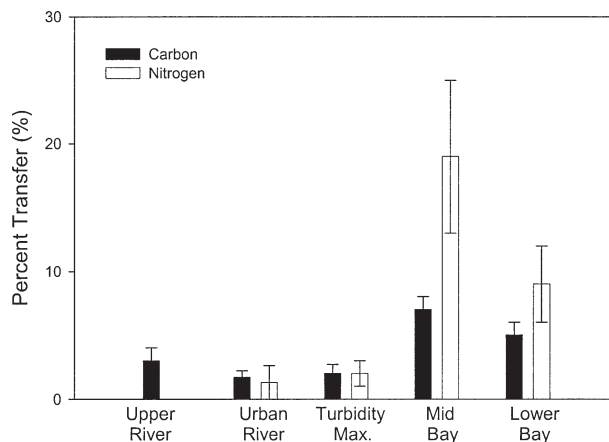


Fig. 7. Transfer of phytoplankton dissolved organic carbon (DOC) and nitrogen (DON) to bacteria for the five regions of the Delaware Estuary. Values are reported as a percentage of the total phytoplankton uptake (e.g., phytoplankton carbon uptake + DOC measured in bacteria) over the course of the experiment.

lower bay regions (Fig. 7). In the upper river, the average percent C transfer was 3% with no measurable N transfer. In the urban river and turbidity maximum regions, C transfer was ca.  $2\% \pm 0.5$  and N transfer was  $1.7\% \pm 1.1$ . In the mid bay C transfer was  $7\% \pm 1.4$ , while N transfer was  $19\% \pm 6.3$ . In the lower bay, C transfer was  $5\% \pm 1.9$  and N transfer was  $9\% \pm 4$ . C transfer rates were less variable at each region of the estuary compared to N transfer, and the magnitude of C transfer, as a percentage of  $>1.0\text{-}\mu\text{m}$  uptake, was lower.

### Discussion

Previous work in the Delaware Estuary has demonstrated a tight seasonal linkage between primary production and bacterial production, indicating that the microbial loop may be a significant pathway for organic matter flow in the estuary (Coffin and Sharp 1987; Kirchman and Hoch 1988; Hoch and Kirchman 1993). The goal of this study was to evaluate the percentage of organic C and N produced in situ that is made available immediately to heterotrophic bacteria, and to understand whether the percentage of organic matter that is transferred from phytoplankton to bacteria varies in either space or time in the estuary, and whether C and N transfer in a similar fashion.

Interpretation of these results requires that phytoplankton and bacteria can be reasonably well separated using simple size fractionation. This approach appears to be appropriate in the Delaware Estuary, where diatom cells dominate the autotrophic community with little picoautotrophic influence (Pennock 1985).

Size fractionation has been employed extensively in previous Delaware Estuary studies to discriminate

between phytoplankton and heterotrophic bacteria (e.g., Coffin and Sharp 1987; Lebo 1990; Hoch and Kirchman 1993; Preen and Kirchman 2004). Hoch and Kirchman (1995) evaluated the validity of using size fractionation (1.0  $\mu\text{m}$  in the estuary, 0.8  $\mu\text{m}$  at the bay mouth and coastal stations) to estimate bacterial rates. Their conclusion was that any contribution made by picoautotrophs to measured bacterial rates was offset by isotope dilution and the loss of bacteria through GF/F filters. They reported measured rates in the <1.0- $\mu\text{m}$  size class as representative of bacterial activity. The present results from chl *a* analysis as well as bicarbonate uptake measurements in the <1.0- $\mu\text{m}$  size class suggest that phytoplankton and heterotrophic bacteria were reasonably well distinguished using the 1.0  $\mu\text{m}$  separation. Comparisons of N uptake rates in the <1.0- $\mu\text{m}$  size fractions of whole community and bacterial control treatments show that, in some cases, N uptake in the <1.0- $\mu\text{m}$  size fraction was enhanced by the presence of >1.0- $\mu\text{m}$  cells. This does not suggest that the two size fractions are composed of the same functional groups (i.e., autotrophs). While it is impossible to achieve an absolute separation of phytoplankton and heterotrophic bacteria using size fractionation techniques, it appears that conditions in the Delaware Estuary make this a reasonable approximation.

The estimates of bacterial production reported here represent only the fraction of bacterial production that is supported by autochthonous DOC supply and does not include any bacterial production supported by allochthonous C input. Even so, it appears that bacterial production supported by autochthonous DOC is comparable to previous estimates of bacterial production in the Delaware Estuary using more traditional techniques. In the present study, autochthonous DOC uptake by bacteria in spring and fall was 9–60 and 0–50  $\text{nmol C l}^{-1} \text{h}^{-1}$ , respectively. Hoch and Kirchman (1993) estimated total bacterial production in the Delaware Estuary in April 1987 using tritiated leucine and thymidine. They estimated production rates of  $20\text{--}80 \times 10^6 \text{ cells l}^{-1} \text{h}^{-1}$ . Converting these rates to C units assuming 20 fg C  $\text{cell}^{-1}$  (Ducklow 2000) results in bacterial C uptake rates of 19–98  $\text{nmol C l}^{-1} \text{h}^{-1}$ . Bacterial production during June and August (14–344  $\text{nmol C l}^{-1} \text{h}^{-1}$ ) of this study was significantly higher than in April and October. Coffin and Sharp (1987) estimated total bacterial production by following the increase in bacterial cell abundance in grazer-excluded experiments. Bacterial production in that study was  $2\text{--}10 \times 10^9 \text{ cells l}^{-1} \text{d}^{-1}$  in August or 45–450  $\text{nmol C l}^{-1} \text{h}^{-1}$ . Interannual variability in bacterial production limits interpretation of these comparisons but it appears that the  $^{13}\text{C}$  uptake rates measured in the present study fall

within the range of previously reported values for bacteria in the Delaware Estuary.

Parallel bacterial production measurements using  $^3\text{H}$ -leucine and  $^{13}\text{C}$  were made during three cruises. Over the whole estuary bacterial uptake of autochthonous DOC represented 100% of total bacterial production. In several cases,  $^{13}\text{C}$  estimates of bacterial production were higher than  $^3\text{H}$ -leucine estimates. The assumed leucine : C is undoubtedly responsible for these results. In general,  $^{13}\text{C}$  estimates of bacterial production were similar to  $^3\text{H}$ -leucine estimates of bacterial production in both the mid and lower bay regions of the estuary where it is thought that the majority of bacterial production is supported by autochthonous production. In the urban river region,  $^{13}\text{C}$  bacterial production values were lower than total bacterial production. This suggests, as was previously interpreted, that bacterial production is more affected by allochthonous DOC in the upper regions of the Delaware Estuary.

Based on the comparison of bacterial C : N uptake ratios, it appears that bacterial uptake of autochthonous DOC is balanced by a combination of direct uptake of inorganic N and autochthonous DON. In the upper regions of the Delaware Estuary, bacterial N uptake was 1.4–1.8 fold higher than the theoretical N demand based on autochthonous C uptake; this results in a C : N uptake ratio of 1.4. Based on bacterial production estimates using tritiated leucine versus  $^{13}\text{C}$ , it appears that bacteria are probably using allochthonous DOC and perhaps allochthonous DON as well as DIN. In the mid and lower bay regions, there is less dependence on allochthonous DOC. Autochthonous DON uptake represented only 23–60% of the theoretical bacterial N demand (based on autochthonous DOC uptake). This resulted in C : N ratios that were considerably higher than the assumed requirements of bacteria (Goldman and Dennett 1991). The apparent N deficiency is supplemented with DIN to achieve C : N uptake ratios close to the Redfield ratio. Bacterial N uptake may, in part, be supported by recycled  $\text{NH}_4^+$  (Jamerlan 1992). These findings are consistent with the findings of Hoch and Kirchman (1995) that as much as 50% of bacterial N demand was supported by  $\text{NH}_4^+$  in the mouth of Delaware Bay in summer. It appears that the previously reported DIN use by bacteria may be in response to allochthonous DOM in the upper estuary and due to high C : N ratio autochthonous DOM in the lower estuary.

While it is clear that bacterial production is dependant on phytoplankton DOM, particularly in the lower regions of the estuary, questions related to understanding what percentage of primary production is routed to bacteria may be evaluated

further. Uptake of autochthonous DOC by bacteria does not appear to follow trends in primary production. Instead, a consistent pattern of low DOC transfer was observed in the upper three regions of the estuary and increased in the mid and lower bay regions. This pattern appeared to hold during all seasons surveyed, including August, where, despite high primary production in the urban river region, the transfer of DOC to bacteria was still low. If it is assumed that there is complete and instantaneous uptake of phytoplankton release products by bacteria then these results suggest that the magnitude of primary production and the magnitude of autochthonous DOM production are not related.

One potentially important source of phytoplankton DOM is extracellular release (ER). Studies of ER have found that the highest rates of release occur under stress such as nutrient limitation (Sharp 1977; Williams 1990; Mykkestad 1995). N or phosphorus limitation has been shown to affect the amount and composition of ER in laboratory cultures (Mykkestad 1995). In those studies, higher absolute rates of release were found in nutrient-limited cultures, and the release products were found to lack the limiting nutrients (i.e., high C : N ratio). Pennock and Sharp (1994) concluded that the mid bay and lower bay regions often experience mild to severe nutrient limitation. The higher rates of autochthonous transfer found for the mid and lower bay regions in this study support the idea that ER may be highest in the nutrient-limited regions of the estuary. In the upper regions of the estuary, where primary production is light limited (Pennock and Sharp 1994), autochthonous DOM transfer appears to be relatively low. Even during the periods with relatively high primary production, autochthonous DOC transfer was ca. 3% of phytoplankton C uptake in the light-limited regions of the estuary. This would suggest that under light limitation, ER in the Delaware Estuary may be a low (1–3%), fixed amount of primary production.

The C : N ratio of autochthonous DOM uptake may provide further insight into phytoplankton DOM production. Pennock (1987) found that in short-term incubations, phytoplankton C : N uptake ratios averaged 34 : 1 (with rates as high as 71 : 1). He concluded that dark N uptake was necessary in order for phytoplankton to achieve balanced (ca. 7 : 1) growth. If there is high autochthonous DOM production during the day, DOM may also have high C : N ratios. The high C : N ratios calculated for autochthonous DOM uptake found in the present study (21 : 1 to 25 : 1) support this idea.

Because percent transfer of autochthonous DOM in the upper regions of the Delaware Estuary is low, there were only two cases when absolute N transfer

was higher than N uptake in the bacterial control. Only during these cases can C : N uptake ratios be calculated for light-limited conditions. The C : N uptake ratios for autochthonous DOM were comparable to the Redfield ratio, suggesting phytoplankton DOM produced under light-limited conditions has a lower C : N ratio compared to cases where phytoplankton DOM is produced under nutrient-limited conditions. The phytoplankton C : N uptake ratios (12 : 1 and 17 : 1 in the urban river and turbidity maximum regions, respectively) were lower than the average for the mid and lower estuary  $28 : 1 \pm 9$ . The lower C : N uptake ratios in phytoplankton, may in part, reflect nutrient sufficiency. The phytoplankton C : N uptake ratios, combined with nonnutrient-limited, autochthonous DOM release, are probably responsible for low C : N ratio uptake by the bacteria.

The apparent difference in the C : N ratio of phytoplankton DOM along the estuarine gradient led to different percentages of C and N transfer to bacteria. In the upper three regions of the estuary, it appears that a small, fixed amount of autochthonous organic C and N (2–4%) is assimilated by bacteria. The similar rates of transfer result in roughly balanced C : N uptake ratios. The bacterial DIN uptake measured may be used to balance C : N ratios in allochthonous supply. In the mid and lower bay regions, the difference in phytoplankton C : N uptake ratios and the C : N ratio in autochthonous DOM uptake result in higher percent transfer of N to bacteria. N transfer rates averaged 9–19% in the mid and lower bay regions with individual rates as high as 50%. Values for percent C transfer were lower, with rates of 5 to 7%. In October C and N transfer were both higher; this was mostly due to low primary production. Despite higher percent N transfer, in absolute terms, C transfer is generally sufficient to maintain the C : N ratio of autochthonous DOM supply well above the Redfield ratio. This requires bacteria to use DIN in order to achieve balanced growth.

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